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Comparitive study of UV-Visible spectrophotometry and high performance liquid Chromatography methods for quantitative estimation of paracetamol in a tablet formulation

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Abstract

The objective of present study was to analyse the comparision between high performance liquid chromatography (HPLC) and UV-Spectrophotometry for quantitative determination of paracetamol in a marketed formulation. Rp-HPLC method involved a reversed-phase XBD C18 column thermostated at 25 °C, UV detection at 230 nm, flow rate of 1.0 ml/min and a mobile phase acetonitrile-water (25:75) was used. These methods showed good linearity over the concentration range of 2–20 µg/mL. The linearity was obtained for paracetamol by reverse phase high performance liquid chromatography (RP-HPLC) R^2=0.993 and by UV - Spectrophotometry R^2=0.977. The precision and recoveries of paracetamol for HPLC and UV-Spectrophotometry methods were in the range of 99.00 -100.10 % and 99.71-100.95 %. Among these two methods high performance liquid chromatography method of analysis showed reliable results for quantitative etermination of paracetamol in a tablet formulation.

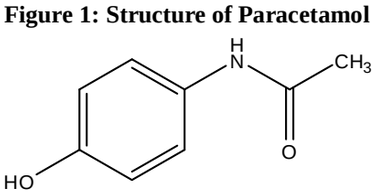
Keywords: Paracetamol, HPLC, UV-Spectrophotometry

1. Introduction

Paracetamol is a pharmaceutical compound widely used as analgesic and antipyretic in the treatment of headaches, pain and as a reliever for fever. Paracetamol (Figure 1) is considered to be the inhibitor of cyclooxygenase (COX), and recent findings suggest that it is highly selective for COX-2. Numerous analytical methods have been documented in the literature for its quantitative determination including spectrophotometry[1], second derivative spectrophotoscopy[2], planar chromatography[3-5], IR spectroscopy[6], capillary chromatography[7,8] and high performance liquid chromatography[9].

Rapid industrial growth and scientific progress increased various synthetic drugs in a market and their utilization made a great demand for fast and economical analytical methods. Hence the purpose of this study was to develop and compare the analytical methods of high performance liquid chromatography (HPLC) and UV-Spectrophotometry for quantitative determination of paracetamol in a marketed formulation.

Figure 1: Structure of Paracetamol



2. Material and Methods

2.1. Reagent and chemicals:

The reference standard of paracetamol(acetaminophen) was obtained from stainlay pharmaceuticals. Whereas, methanol and acetonitrile (HPLC) were obtained from merk. Milli –Q ultra pure purification system provides Grade-1 water (Millipore, USA) 40 mg/ tablet was punched from local marketed paracetamol tablet and is labeled.

2.2. Instruments and analytical conditions:

On Agilent 1200 system composed of quarternary pump, UV detector and HP chemstation software the HPLC analysis were carried out. Symmetry C_{18}(250 mm ×4.6 i.d 5µm partical size) column was used. Which is maintained at 40^0C. at 230 nm UV detection was performed. Acetonitrile and water (25:75% v/v) at flow rate 1mL min^{-1} are the mobile phases used. The volume of injection was 4µL. Having a path length of 1cm Quartz cuvette, UV- spectrophotometric analysis was carried out on Perkin Elmer Lambda 650 UV/visible spectrophotometer.

2.3. Preparation of standard and reference solution

2.3.1. Preparation of Standard Stock Solution

Weigh accurately about 40 mg of pure authentic sample (standard) of paracetamol and transferred to 500 ml flask. To ensure complete solubilization, 0.5N NaOH (1mL) was added and the solution was diluted with distilled water up to required volume. Aliquots from each working solution were combined and diluted with mobile phase to yield a solution with final concentrations of 2,4,6,8,10,12,14,16,18,20, µg mL^{-1}. The stock solutions were filtered through a 0.45 µm nylon membrane followed by sonicate for five minutes.

2.3.2. Paracetamol sample solution

Ten tablets of paracetamol were weighed out and powdered. The powdered sample equivalent to 40 mg of paracetamol was accurately weighed out and then transferred to 500 ml flask. 0.5N NaOH (1mL) was added for complete solubilization which was diluted to mark with distilled water.

2.4. Method validation

2.4.1. Limit of quantitative (LOC) and Limit of detection (LOD)

A series of diluted standard paracetamol solutions were analyzed by both HPLC and UV visible spectrophotometer for measuring LOC and LOD. By HPLC method, Limit of detection for paracetamol was calculated at the signal to noise ratio of 3 and was found to be 0.829 µg mL^{-1}. Quntification limit of paracetamol was calculated at signal to noise ratio of 10 and was found to be 2.763 µg mL^{-1}. For UV visible spectrophotometry, limit of detection and quantification was calculated and was found to be 4 µg mL^{-1} and 13.3 µg mL^{-1} respectively.

2.4.2. Linearity

By analysis of working standard solutions of ten (10) different concentrations (n= 3) linearity of

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HPLC method was elevated. Y= 0.091x (r^2= 0.997, n = 10) was the regression equation obtained 2 to 20 µg mL^{-1} was the range of linearity.

The range from 4 to 18 µg mL^{-1}, linearity was calculated for UV visible spectrophotometry. By obeying Beer's law with coefficient of correlation r^2 of 0.997 the plot was linear in range of 4 to 10 µg mL^{-1}. Beer's law negative deviation is beyond 10 µg mL^{-1}. Q- test was applied at 90% confidence interval points 10 µg mL^{-1} were removed from fig- 2 and a linear plot was obtained as shown in fig-3.

Fig.2: Plot for standard paracetamol on UV-Visible spectrophotometer showing deviation from Beer's law

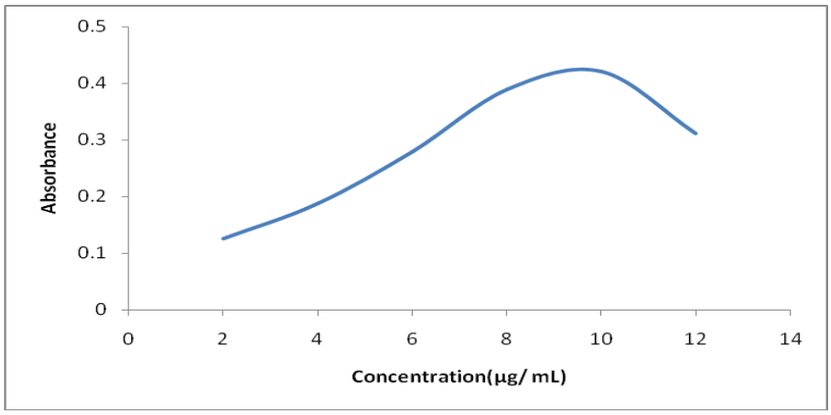
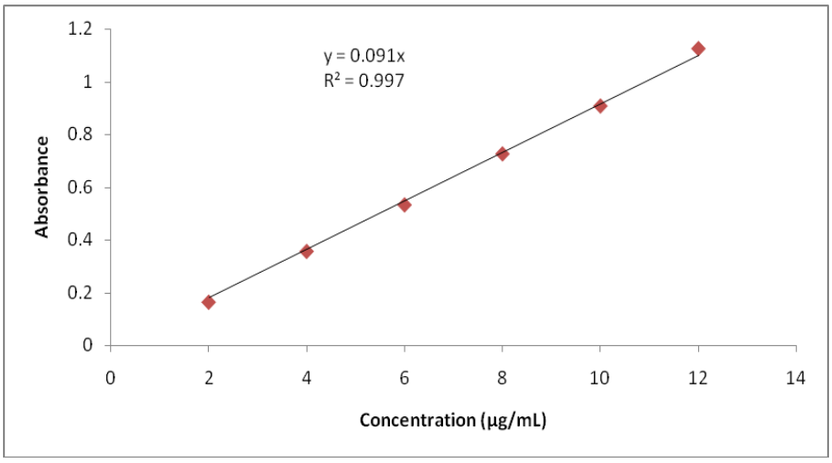


Fig. 3: Plot showing linear relation between absorbance and concentration by applying Q-test



2.4.3. Accuracy: Reference standard solutions of paracetamol at a concentration of 4, 6 and 8 µg mL-1 were added to the tablet formulation for the determination of method of accuracy. Before the preparation of calibration curve, the sample solutions were prepared freshly and analyzed for three consecutive days by using HPLC and UV visible spectrophotometry. The results for accuracy from these two methods are depicted in the table 2.

2.4.4. Precision: The intra-day precision was determined by analyzing sample solutions of three different concentration 4, 6 and 8 µg/ml (n = 3) using the UV and HPLC methods. The intermediate precision (inter-day variation at same concentration) was examined for three consecutive days (n 3 = 3). Paracetamol content and relative standard deviations (R.S.D) for both UV and HPLC 4 methods were calculated and are summarized in Table 2.

2.5. Analysis of paracetamol tablets

Samples of paracetamol tablets were analyzed by HPLC and UV methods. Before the analysis the tablets were weighed and finely powdered. The paracetamol contents were determined using the two methods and the obtained results were statistically proved using test hypothesis at 0.01 significance level.

3. Results and discussion

Different chromatographic conditions were tested by modifying the composition of mobile phase, flow rate and column. Finally, applying mobile phase of water: acetonitrile in a ratio of (75:25v/v) at a flow rate of 1 mL min-1 showed more adequate results. So, using this combination of mobile phase at pH 7.4 and a C18 column, an adequate peak symmetry (symmetry=0.5) and short run time of 3 min was achieved as shown in Fig.4.

In UV-Visible spectrophotometry standard paracetamol sample showed absorption band at 230 nm (Fig 5). So wavelength of 230 nm was selected for absorption measurement of paracetamole content in tablets.

Fig. 4: Chromatogram obtained for paracetamol sample solution at 4µgmL^{1} using symmetry C18 13 column at 400C and mobile phase composed of water and Acetonitrile (75:25% v/v) at flow rate 14 of 1mLmin-1. Detection was performed at 230 nm.

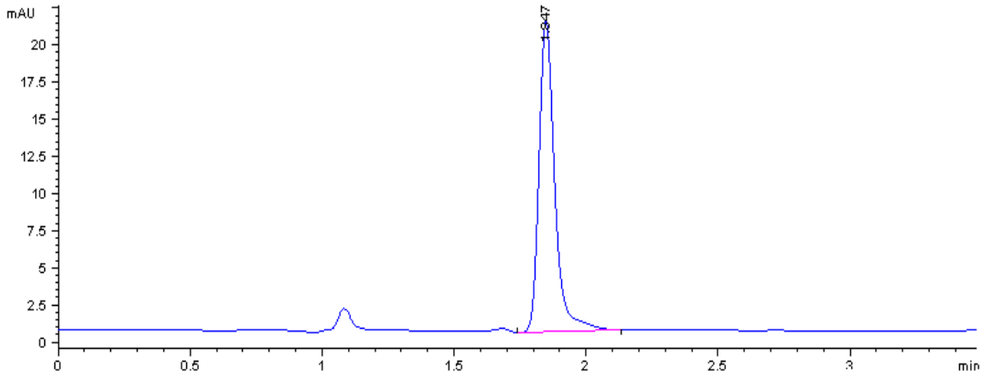
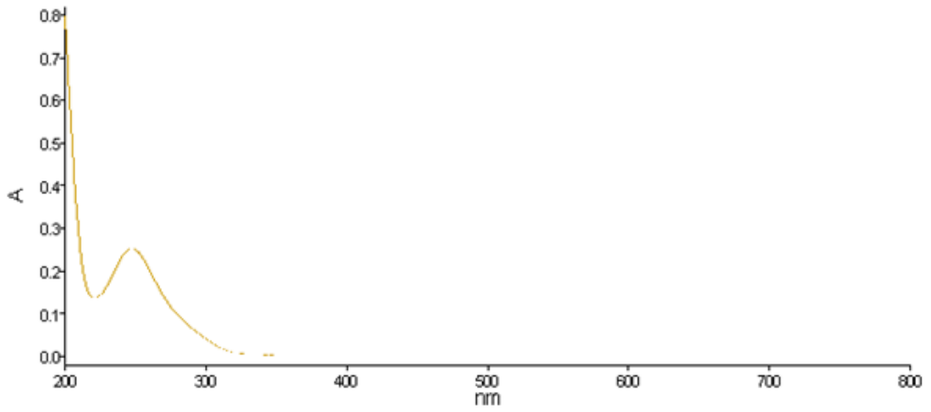


Fig. 5 UV Spectrum of paracetamol sample solution at 6µgmL^{-1}, in water



3.1. Validation

A linear relationship was found between paracetamol concentration and response time of both HPLC and UV methods. Table 1 shows regression analysis data for both the methods. HPLC shows regression coefficient of 0.993 while UV method shows regression coefficient value of 0.9772 which is little lower than HPLC method.

The precision data obtained for the two methods are tabulated in table 2. Both HPLC and UV-visible spectrophotmetric method showed R.S.D values lower than 2% presenting good precision however, HPLC method was highly precise than UV method.. Accuracy was investigated by means of % recovery (n=3) experiments using developed methods. Both chromatographic and spectrophotometric methods exhibited recoveries close to 100%, best recovery was achieved by HPLC-method.

The LOD and LOQ for chromatographic method were obtained by considering signal to noise ratio of 3 and 10 and were found to be 0.829µgmL-1 and 2.76µgmL-1 respectively. For UV-method LOD and LOQ were found to be 1.25 µgmL-1 and 3.51 µgmL -1 respectively. HPLC proved to be more sensitive method, compared to UV method.

3.2. Analysis of paracetamol content in tablets:

By using HPLC and UV-visible spectrophotometer the quantitative analysis of paracetamol in tablets was carried out and is mentioned in table-3. HPLC method showed slightly lower % R.S.D than UV method. Since, both methods showed nearly same amount of paracetamol content in tablets, both analytical techniques are employed for quantitative analysis.

Table 1: Overview of the linearity data obtained for paracetamol by chromatographic and spectrophotometric methods.

Regression parameters	HPLC	UV
Regression Coefficient(r2)	0.993	0.977
Slope ± Standard Error	11.619±0.344	0.0455±0.004909
Intercept± standard Error	-9.6334±4.27	-0.0024±0.03607
Standard Error of Estimate (Standard deviation of regression)	6.27	0.021952
Concentration Range (µgmL-1)	2-20	4-10
Number of Points	10	4

Table 2: Validation Parameters of the elevated methods for Paracetamol determination

Validation Parameter	HPLC	UV
Intra-day Precision n=3 (R.S.D %)	0.332	0.452
Inter-day Precision n=3(R.S.D %)	0.281	0.556
Accuracy n=3 (Mean % Recovery)	99.71-100.95	99.00-100.10
LOD (µgmL-1)	0.829	1.25
LOQ (µgmL-1)	2.76	3.51

Table 3: Quantitative analysis of paracetamol content in commercially available tablets by our proposed methods

Drug	Claimed concentration (mg/tablet)	Factors (n =5)	HPLC	UV
Paracetamol	40	Mean (%)	99.95	97.89
		Amount found (mg)	39.99	39.99
		S.D	0.3501	0.4511
		% R.S.D	0.3503	0.4521

4. Conclusion

It can be concluded from our work that quantitative analysis of paracetamol in tablets and raw material were studied and compared by both HPLC and UV methods with sensitive, accurate and precise results. Both these methods can be used for quantitative determination of paracetamol but HPLC method is most effective and reliable.

References

[1] Behera S, Ghanty S, Ahmad F, Santra S, Banerjee S. UV-Visible Spectrophotometric Method Development and Validation of Assay of Paracetamol Tablet Formulation. *J Anal Bioanal Tech* 2012; 3:1-6.

[2] Chakraborthy B, Lakshmi Sivasubramanian, Anusha. Simultaneous estimation of Paracetamol, Cetrizine and Dextromethorphan using second derivative spectrophotometry. *Int J Pharm Pharm Sci* 2014; 6(4):291-3.

[3] Roy J, Saha P, Sultana S, Kenyon AS, Rapid screening of marketed paracetamol tablets: use of thin-layer chromatography and a semiquantitative spot test. *Bull World Health Organ* 1997; 75(1): 19–2

[4] Dimitrovska A, Trajkovic-Jolevska S, Nancovska A, Ilievska M. Determination of propyphenazone, paracetamol, caffeine and codeine phosphate with thin layer chromatography. *Bull Chem Technol Macedonia* 1995; 14:39–1.

[5] Tavallali H, Zareiyan SF, Naghian M. An efficient and simultaneous analysis of caffeine and paracetamol in pharmaceutical formulations using TLC with a fluorescence plate reader. *Journal of AOAC International* 2011; 94(4):1094–9.

[6] Khanmohammadi M, Garmarudi AB, Moazzen N, Ghasemi K, Qualitative Discrimination Between Paracetamol Tablets Made by Near Infrared Spectroscopy and Chemometrics With Regard to Polymorphism. *J Struct Chem* 2010; 51(4): 663-9.

[7] Dana N.Haj-Ali, Imad I. Hamdan. Development of a capillary electrophoresis method for the determination of orphenadrine citrate in tablets in the presence of paracetamol. *Saudi Pharm J* 2010; 18(4): 233–7.

[8] Yao R, Xu Q, Du L. Direct determination of four components in compound paracetamol and diphenhydramine tablets by wide bore capillary gas chromatography. *Se Pu* 2007; 25(2):258-1.

[9] Phazna Devi TA, Aravind Setti, Srikanth S, Sivaramaiah Nallapeta, Smita C. Pawar, Venkateshwara Rao J. Method development and validation of paracetamol drug by RP-HPLC. *J Med Allied Sci* 2 0 1 3; 3(1):0 8 - 4.